



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re application of:

Chatterjee, D.K.

Appl. No. 09/558,421

Filed: April 26, 2000

For: **Mutant DNA Polymerases and
Uses Thereof**

Art Unit: 1652

Examiner: Rao, M.

Atty. Docket: 0942.3600003/RWE/BJD

Declaration of A. John Hughes, Jr.

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, A. John Hughes, Jr., do hereby declare and say:

1. THAT, I, A. John Hughes, Jr., hold the degree of Ph.D. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

2. THAT, since 1991, I have been employed by Life Technologies, Inc. (LTI) (and now Invitrogen Corporation)¹, the assignee of the above-captioned application, in the capacity of Scientist. See Exhibit A.

¹Life Technologies, Inc. merged with Invitrogen Corporation on September 12, 2000, with Invitrogen Corporation being the surviving entity.

3. THAT, during my employment by LTI (and now Invitrogen Corporation), I worked with Dr. Deb K. Chatterjee on a project involving the cloning, expression, and characterization of wild-type and mutant DNA polymerases.

4. THAT, I have reviewed my laboratory notebooks detailing my work on the project. Based on these laboratory notebook records and my recollection, the following activities involving my work, and relating to the DNA polymerase project, took place during the period from about October 16, 1994, until about September 8, 1995.


On or about February 1, 1995, I performed a quality control experiment on an aliquot of *Tne* polymerase. I determined the unit activity concentrations of two *Tne* polymerase samples to insure their quality. This activity was recorded on page 51 of notebook 3875. A copy thereof is attached as exhibit 1.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom.

Further, declarant sayeth not.

Date: 11/27/01

Name: A. John Hughes, Jr.

Signature: 

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SKGF1/25/98 dcw

A. John Hughes, Jr., Ph. D.
41320 Rue-Jadot
Temecula, California 92591
(909) 308-9220

ACHIEVEMENTS:

Graduated *magna cum laude*, College of the Albemarle.
Cum laude graduate, East Carolina University.
Vice President, American Chemical Society Student Affiliates, East Carolina University.
Delbert A. Greenwood Award, Outstanding Biochemistry Graduate Student, Brigham Young University.
National Institute of Health Postdoctoral Fellowship, 1988-1990.
1997 Life Technologies, Inc. award for Leadership Excellence.
1998 Life Technologies, Inc. award for Leadership Excellence.
David L. Coffin award for technical innovation, 1998.
Life Technologies Presidential award 1998.

INVITED MEMBERSHIPS:

Phi Kappa Phi National Honor Society
Phi Theta Kappa National Honor Society

EDUCATION:

Ph. D. - Department of Chemistry, Brigham Young University, Provo Utah, June 1987.
Dissertation: "Biochemistry and Synthesis of Dinucleoside Tetraphosphates and their Analogs".
Minor: Organic Chemistry.

B. S. - Biochemistry, East Carolina University, Greenville North Carolina, May 1982.

A. S. - College of the Albemarle, Elizabeth City, North Carolina, May 1980.

WORK EXPERIENCE:

Senior Principal Scientist – Invitrogen Life Technologies, Inc., Manufacturing, Carlsbad California, August 2001 – Present.

Principal Scientist - Life Technologies, Inc., Technical Manufacturing, Frederick Maryland, January 1997 – August 2001. Direct large-scale protein purification development including cGMP processes. Work in a team environment to help design cGMP process facility, including directing efforts of facility and process engineering, design and execution of factory acceptance tests, installation qualification, operation qualification and performance qualification of all purification process equipment, review and write documentation. Work with and train process

operators. Provide site technical leadership.

Senior Scientist – Life Technologies, Inc., Technical Manufacturing, Frederick Maryland, June 1995 – December 1996. Project leader. Direct large scale protein purification projects for the operations unit. Oversee technical change control for purification protocols. Write and review process documentation, including protocols, batch records, SOP's, and QC documents. Interact with external customers. Provide site technical leadership and training.

Senior Scientist - Life Technologies, Inc., Molecular Biology R&D, Gaithersburg Maryland, April 1993 – May 1995. Project leader. Direct research activities of the Enzymes section of the Enzymes and Genetic engineering Group. Serve as a principal resource for purification development, and implementation of ISO 9000. Participate on interdisciplinary teams as a resource in the area of protein research. Write patents and articles including technical bulletins. Responsible for training of new scientists and student interns.

Staff Scientist - Life Technologies, Inc., Molecular Biology R&D, Gaithersburg Maryland, February 1991 - March 1993. Isolation and characterization of DNA modifying and restriction enzymes from mesophilic and thermophilic sources. Development of large-scale purification procedures for production. Assay development for evaluation of thermophilic polymerases. Fidelity studies, and enzyme kinetics.

Research Scientist - SYNTEX Pharmaceuticals, Boulder Colorado, November 1990 to February 1991. Short-term contract work to develop a novel immobilized enzymic activity to carry out a stereospecific synthetic step in the production of a proprietary drug. Evaluation and purification of enzymes, assay development and covalent cross-linking of protein to solid support. Direct research of laboratory technicians.

Postdoctoral Fellow - University of Colorado Health Sciences Center, Denver, Colorado, July 1987 to November 1990. Developed purification schemes for *E. coli* DNA polymerase III holoenzyme subassemblies and recombinant subunits. Performed nucleotide interaction studies. Characterized novel subunit interactions with *E. coli* DNA polymerase II.

PUBLICATIONS:

Bary Westfall, Kala Sitaraman, Joseph Solus, A. John Hughes, Jr., and Ayoub Rashtchian, (1997) *FOCUS* vol 19, 46.

Nicoletta Sacchi, Luisa Schiaffonati, Ivana Magani, Carmela Pappalardo, A. John Hughes, Jr., Marlene Darfler and Andre T. Hoogeveen; "Detection and subcellular localization of an AML1 chimeric protein in the t(8;21) positive acute myeloid leukemia" (1996) *Oncogene* 12, 437-444.

Deborah A. Polayes and A. John Hughes, Jr.; "Efficient Protein Expression and Simple Purification Using the pProEX-1™ System" (1994) *FOCUS* vol. 16 No. 3, pp.81-84.

Deborah A. Polayes, Adam Goldstein, Gary Ward, and A. John Hughes, Jr.; "TEV Protease, Recombinant: A Site-Specific Protease for Efficient Cleavage of Affinity Tags From Expressed

Protiens" (1994) *FOCUS* vol. 16 No. 1, pp. 2-5.

John Harding, Marleen Darfler, Alberto Haces, Ray Hadley, A. John Hughes Jr., Roger Lasken and Paul Watkins "Synthesis of Fluorescent DNA for Sequencing by Single Molecule Detection and for *in situ* Hybridization" (1992) *Genome Science and Technology* **A40**, 13.

Carol A. Wu, Ellen L. Zechner, A. John Hughes, Jr., Mary A. Franden, Charles S. McHenry, and Kenneth J. Marians "Coordinated Leading-strand Synthesis at the *Escherichia coli* DNA Replication Fork" *J. Biol. Chem.* (1992) **267**, 4064-4073.

A. John Hughes, Jr., Deb K. Chatterjee, Roger S. Lasken, James H. Campbell, and Gary F. Gerard "Expression, Purification and Characterization of an *Escherichia coli* Phage T5 DNA polymerase 3'-5' Exonuclease Mutant" *J. Cell. Biochem.* (1992) **16B**, 84.

Carol A. Wu, Ellen L. Zechner, A. John Hughes, Jr., Mary A. Franden, Jo Anna Reems, Charles S. McHenry, and Kenneth J. Marians "Coordinated Leading-strand Synthesis at the *Escherichia coli* DNA Replication Fork" *J. Cell. Biochem.* (1992) **16B**.

A. John Hughes, Jr., Sharon K. Bryan, Hong Chen, Robb E. Moses, and Charles S. McHenry "*Escherichia coli* DNA Polymerase II Is Stimulated by DNA Polymerase III Holoenzyme Auxiliary Subunits" *J. Biol. Chem.* (1991) **266**, 4568-4573.

PATENTS:

John Hughes, Jr.; "Nucleic Acid -free reverse transcriptase and methods of production thereof", 2000 (pending).

A. John Hughes, Jr., and Deb K. Chatterjee; "Cloned DNA polymerases from *Thermatoga* and mutants thereof", No. 6,015,668, 2000.

Adam Goldstein and A. John Hughes, Jr.; "Nucleic Acid-free thermostable enzymes and methods of production thereof", No. 5,861,295, 1999.

Deb K. Chatterjee, and A. John Hughes, Jr.; "Cloned DNA polymerases from *Thermatoga neapolitana*", No. 5,912,155, 1999.

A. John Hughes, Jr., and Deb K. Chatterjee; "Cloned DNA polymerases from *Thermatoga neapolitana* and mutants thereof", No. 5,939,301, 1999.

Ilse Blumentals, Roger S. Lasken, Brian Schmidt, Mary Longo, A. John Hughes, Jr., and Deb K. Chatterjee; "Thermostable DNA polymerase from *Desulfurococcus* Tok 12-s1" 1995.